Draft Guidance for Industry and Food and Drug Administration Staff

Class II Special Controls Guidance Document: Nucleic Acid-Based *In Vitro* Diagnostic Devices for the Detection of *Mycobacterium tuberculosis* Complex in Respiratory Specimens

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Devices and Radiological Health
Office of *In Vitro* Diagnostic Device Evaluation and Safety

Preface

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Class II Special Controls Guidance Document: Nucleic Acid-Based *In* V*itro* Diagnostic Devices for the Detection of *Mycobacterium tuberculosis* Complex in Respiratory Specimens

1. Introduction

This document was developed to support the reclassification of nucleic acid-based *in vitro* diagnostic devices for the detection of *Mycobacterium tuberculosis* complex (MTB-complex) in respiratory specimens from class III into class II. These devices are intended to be used as an aid in the diagnosis of pulmonary tuberculosis.

When finalized, designation of a guidance document as a special control means that any manufacturer intending to market nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex in respiratory specimens will need to address the issues covered in the special controls guidance. The firm will need to show that its device addresses the issues of safety and effectiveness identified in the guidance, either by meeting the recommendations of the guidance or by some other means that provides equivalent assurances of safety and effectiveness.
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2. Background

Tuberculosis is a bacterial infection caused by *Mycobacterium spp*. Pulmonary tuberculosis is the most common clinical presentation of tuberculosis in adults although extra-pulmonary disease is more prevalent in children. Pulmonary tuberculosis can occur following exposure to a person with active tuberculosis. Infection with *Mycobacterium tuberculosis* is the most common cause of pulmonary tuberculosis. Although infection with any member of the MTB-complex[1] can lead to pulmonary tuberculosis, *M. bovis* is the cause of active pulmonary tuberculosis in less than 2% of subjects in the US [Ref. 1], and members of MTB-complex other than *M. bovis* and

[1] *M. tuberculosis* complex includes the following species: *Mycobacterium tuberculosis, M. bovis, M. africanum, M. canetti, M. microti, M. caprae,* and *M. pinnipedi*.

M. tuberculosis are even less common causes of disease. Transmission of the organism to the new host occurs by inhalation of airborne particles released from the actively infected individual.

Pulmonary tuberculosis may be active or latent. Most people who are infected with tuberculosis harbor the tuberculosis bacterium but are asymptomatic. This is known as latent tuberculosis. In some people, this organism overcomes the defenses of the immune system and begins to multiply, resulting in the progression from latent tuberculosis (TB) infection to active TB disease. Some people develop active TB disease soon after infection, while others develop active TB disease later when their immune system becomes weak. Overall, there is a 5-10% risk for patients with TB infection to develop active TB disease; however, the risk varies due to many factors, and may be substantially increased by immunosuppression. [Ref.2]

The incidence of active tuberculosis in the United States (US) has declined steadily since 1953, although an increase was seen from 1989–1992, attributed to an increase in HIV infected subjects. [Ref.3] Estimates of active tuberculosis in the US in 2010 were 3.6 cases/100,000 population, with approximately 60% of the active TB cases in the US are imported, i.e., identified among foreign-born persons. [Ref.3]

FDA concludes that special controls, when combined with the general controls Federal Food, Drug & Cosmetic Act (the FD&C Act), will be sufficient to provide reasonable assurance of the safety and effectiveness of nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex in respiratory specimens. Designation of this guidance document as a special control means that a manufacturer who intends to market a device of this type should (1) conform to the general controls of the FD&C Act, including the premarket notification requirements described in 21 CFR 807 Subpart E, (2) address the specific issues of safety and effectiveness identified in this guidance document, and (3) obtain a substantial equivalence determination from FDA prior to marketing the device.

This draft guidance document identifies the proposed classification regulation and associated product codes for nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex in respiratory specimens (refer to Section 3). In addition, other sections of this draft guidance document list the risks to health and describe measure that, if followed by manufacturers and combined with the general controls, will generally address the risks associated with these devices and will lead to a timely premarket notification [510(k)] review. This draft document, when final, will supplement other FDA documents regarding the specific content requirements of a premarket notification submissions for nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex in respiratory specimens. You should also refer to 21 CFR 807.87 and the Center for Devices and Radiological Health (CDRH) Device Advice at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm.

3. Scope

The scope of this document is limited to the devices identified and classified under 21 CFR 866.3372 (product code MWA):

21 CFR 866.3372 – Nucleic acid-based in vitro diagnostic devices for the detection of *Mycobacterium tuberculosis* complex in respiratory specimens.

(a) *Identification*. Nucleic acid-based in vitro diagnostic devices for the detection of *Mycobacterium tuberculosis* complex in respiratory specimens are qualitative nucleic acid-based in vitro diagnostic devices intended to detect *M. tuberculosis* complex nucleic acids extracted from human respiratory specimens. These devices are non-multiplexed and intended to be used as an aid in the diagnosis of pulmonary tuberculosis when used in conjunction with clinical and other laboratory findings. These devices do not include devices intended to detect the presence of organism mutations associated with drug resistance. Respiratory specimens may include sputum (induced or expectorated), bronchial specimens (e.g., bronchoalveolar lavage or bronchial aspirate), or tracheal aspirates.

This draft special controls document includes recommendations for satisfying the proposed requirement of special controls for all devices of this type. This draft special controls document is not intended to address devices for the detection of MTB-complex antigens, for assessment of host serological or immunological responses to MTB-complex infection, or for non-respiratory specimen types. This draft guidance is also not intended to make recommendations for establishing the performance of multiplexed nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex in respiratory specimens along with other pathogens or other intended uses such as the presence of mutations associated with drug resistance. Those seeking guidance for devices that fall outside of the scope of this draft special controls document should contact the Division of Microbiology Devices.

4. Risks to Health

FDA has identified the risks of false negative and false positive test results, which can lead to individual and/or public health consequences, as risks to health associated with this device that require special controls. In addition, FDA has identified biosafety risks to healthcare workers handling specimens and control materials with the possibility of transmission of tuberculosis infection to healthcare workers as a risk requiring special controls. These risks, and the location of recommendations for mitigating them, are summarized in Table 1 below.

Failure of nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex in respiratory specimens to perform as indicated or an error in interpretation of results may lead to misdiagnosis and improper patient management or to inaccurate epidemiological information that may contribute to inappropriate public health responses. A false positive result may lead to: incorrect treatment of the individual with possible adverse effects, unnecessary patient isolation and/or other human contact limitations, unnecessary patient and general public distress, and unnecessary investigations of patient contacts. A false negative result may lead to disease progression and the risk of transmitting disease to contacts and the general public.

We recommend that manufacturers who intend to market a device of this type conduct a risk analysis prior to submitting a premarket notification to identify any other risks specific to their device. The premarket notification should describe the risk analysis method used. If you elect to

use an alternative approach to mitigate a particular risk identified in this draft guidance document, or if you or others identify additional potential risks from use of a device of this type, you should provide sufficient detail regarding the approaches used to mitigate these risks.

Table 1 – Identified Risks and Recommended Mitigation Measures

Identified Risks	Recommended Mitigation Measures
False positive test results may lead to incorrect treatment of the individual with possible adverse effects. The patient may be subjected to unnecessary isolation and/or other human contact limitations. Unnecessary contact investigations may also occur.	Section 5 (Device Description) Section 6 (Performance Studies) Section 7 (Labeling)
False negative test results could result in disease progression and the risk of transmitting disease to others.	Section 5 (Device Description) Section 6 (Performance Studies) Section 7 (Labeling)
Biosafety risks to healthcare workers handling specimens and control materials with the possibility of transmission of tuberculosis infection to healthcare workers	Section 7 (Labeling)

5. Device Description

You must ensure your device description meets the requirements of 21 CFR 807.87. Your 510(k) submission must include proposed labels, labeling, and advertisements sufficient to describe the device, its intended use, and the directions for its use (see 21 CFR 807.87(e)). Further, the proposed labeling submitted for your device must satisfy the requirements in 21 CFR 809.10(a) and (b). In satisfying 21 CFR 807.87 (e) and 21 CFR 807.87(f), you should include the intended use, the specimen types that can be used with the device, the technological characteristics of the device, the relevant regulation, appropriate product code, and legally marketed predicate device that you will compare with your device. In order to quickly view all aspects of your device compared with the predicate, we recommend that you include a table outlining the similarities and differences between the predicate and the new device. The following subsections describe the descriptive information that should be included in a premarket submission in greater detail. Additional information about the content of a 510(k) can be found on the FDA website, Device Advice at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/PremarketNotification510k/ucm142651.htm#compare.

a. Intended Use

You should clearly state the clinical indications for which the test is to be used, the specimen types and the specific population for which the test is intended. You should include a description of the patient populations (e.g., gender, age, symptoms) for whom the test is intended if there are

possible limitations to device use. Your intended use statement should also specify that the test is qualitative, and that it is to be used as an adjunct to other laboratory tests and clinical findings. In addition you should include the following statement immediately below the intended use statement:

"The [Insert name of test] test should only be performed in laboratories that follow safety practices according to the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories[2] and/or applicable state or local regulations. The [Insert name of test] test must be performed in conjunction with mycobacterial culture to address the risk of false negative results and to recover the organisms for further characterization and susceptibility testing."

b. Test Methodology

You should include the following elements of test methodology, as applicable:

1. The specific test methodology to be used, e.g., real-time PCR, transcription mediated amplification, nucleic acid-based signal amplification,
2. Additional information regarding assay oligonucleotides, including:
    - The rationale for the oligonucleotides selected, including a listing of the specific sequences used. Assay oligonucleotides are the main components of a nucleic acid based detection system, and the performance of the assays is highly dependent on the quality of these reagents.
    - The specificity of these oligonucleotides in distinguishing MTB-complex and any information indicating the potential for non-specific binding.
    - Justifications for alignments made to generate consensus sequences and/or best-fit modifications made to existent sequences, e.g., to permit maximum homology to several strains.
    - Information on size, guanine-cytosine content, melting temperatures, hairpin or other secondary structures if any, and the nucleotide position on the genome map of the assay oligonucleotides.
3. Assay procedural steps, e.g., pipetting, incubation, washing, and mixing.
4. Methods for collection and handling of each specimen type.
5. Reagent components provided or recommended for use, and their function within the system, e.g., solid support, buffers, fluorescent dyes, chemiluminescent reagents, substrates, conjugates, other reagents.
6. Illustrations or photographs of the device and any non-standard equipment or methods as appropriate.

When applicable, your submission should describe the device design control specifications that address or mitigate risks, such as false positive results due to sample contamination, associated with nucleic acid-based procedures for detecting MTB-complex. Design control specification should include the following:

[2] http://www.cdc.gov/biosafety/publications/bmbl5/index.htm

1. Positive, negative, and inhibition controls to ensure accurate test results.
2. The validated/recommended methods for nucleic acid extraction to be used with the reagents specified for the test system. Validated extraction method(s) should be described for each of the different specimen types included in the device intended use statement.
3. The methods for optimizing the reagents and test procedure for recommended instruments.
4. Design features for minimizing risks of exposure to health care personnel.

Your 510(k) submission should provide performance information that supports the conclusion that device design requirements have been met.

c. Instrumentation – Hardware and Software

In your 510(k) submission, you should include all software information in accordance with the level of concern described in the FDA guidance document entitled "Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices."[3] The level of concern should be driven by the hazard analysis in the absence of mitigations, regardless of the effects of the mitigations on the individual hazards. The level of concern determined for the software device should be stated in the submission. The level of concern of nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex in respiratory specimens is typically Moderate. Software flaws could potentially result in patient injury if inaccurate test result information is given to the healthcare provider and the patient.

Your 510(k) submission should include a clear description of how raw signals are converted into a result, including adjustment to the background signal for normalization. In addition, you should describe software controls for identifying and managing anticipated problems.

You should include the following documentation for software development and implementation in your 510(k) submission:

- System and Software Requirements
- Hazard Analysis
- Architecture Design Chart
- Software Design Specification
- Software Development Environment Description
- Verification and Validation
- Traceability Analysis
- Unresolved Anomalies (do the anomalies influence safety and effectiveness)

Configuration of the hardware and software components should be very similar or identical to that anticipated for the final version of the device before beginning clinical studies. If any

[3] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089543.htm

significant changes are made to the hardware or software after the completion of the clinical studies but before the clearance and distribution of the device, you should perform a risk assessment and include it in your 510(k) submission.

The following references may aid in the development and maintenance of a new device under good software life cycle practices consistent with FDA regulations:

- The guidance entitled "General Principles of Software Validation; Final Guidance for Industry and FDA Staff" (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm085281.htm);
- The guidance entitled "Off-the-Shelf Software Use in Medical Devices" (http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm073779.pdf);
- 21 CFR 820.30 Subpart C – Design Controls of the Quality System Regulation (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?FR=820.30);
- ISO 14971-1; Medical devices - Risk Management - Part 1: Application of Risk Analysis;
- AAMI SW68:2001; Medical Device Software - Software Life Cycle Processes

For instruments and systems that measure multiple signals, and other complex laboratory instrumentation, we recommend that you consult with the Division of Microbiology Devices.

d. Controls

For nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex in respiratory specimens, we recommend that you include the controls described below in your 510(k) submission. You should define the source of the controls and have a plan for their continued renewal. When conducting the performance studies described (see Section 6), we recommend that you run external controls, using the appropriate specimen type, every day of testing for the duration of the analytical and clinical studies.

1) Negative Controls

Blank or no template control

The blank, or no-template control, should contain buffer or specimen transport media and all of the assay components except target nucleic acid. These controls are used to rule out contamination with target nucleic acid or increased background in the amplification reaction. It may not be applicable for assays performed in single test disposable cartridges or tubes.

Negative control

The negative sample control contains non-target nucleic acid or, if used to evaluate extraction procedures, a complete non-MTB-complex organism. Negative results for this control confirm that that signals are not obtained in the absence of target sequences, e.g., due to non-specific priming or detection. Examples of acceptable negative sample control materials include:

- Specimens from MTB-complex nucleic acid negative individuals.

- Specimens containing a non-target mycobacterial organism (e.g., MTB-complex nucleic acid negative specimens spiked with *Mycobacterium gordonae*)
- Surrogate negative control, e.g., non-target encapsulated nucleic acid.

2) Positive Controls

Positive control for complete assay

The positive control contains target nucleic acids, and is used to test the entire assay process, including sample lysis or nucleic acid extraction, amplification, and detection. It is designed to mimic a patient specimen and is run as a separate assay, concurrently with patient specimens, at a frequency determined by a laboratory's Quality System (QS). One example of an acceptable positive assay control is MTB-complex strains/isolates containing target sequences detected by the device.

Positive control for amplification/detection

The positive control for amplification/detection contains purified target nucleic acid at or near the limit of detection for a qualitative assay and is not usually taken through the sample lysis or nucleic acid extraction process. It verifies the integrity of the reaction components and instrument when negative results are obtained. It also indicates that the target can be detected if it is present in the sample lysates or extracted sample. Examples of this type of control include a non-infectious DNA plasmid containing the target sequence, or purified full length double stranded genomic DNA from an MTB-complex isolate.

3) Internal Control

The internal control is a non-target nucleic acid sequence that is co-extracted and/or co-amplified with the target nucleic acid. It controls for integrity of the reagents (polymerase, primers, etc.), equipment function (thermal cycler), and the presence of inhibitors in the specimen. Examples of acceptable internal control materials include:

- Human nucleic acid co-extracted with MTB-complex and primers amplifying human housekeeping genes (e.g., RNaseP, β-actin).
- A non-infectious DNA plasmid containing the non-target nucleic acid that is added to the sample either prior or after sample lysis/extraction.
- A complete non-MTB complex organism that is added to the sample prior to sample lysis/extraction.

The need for this control is determined on a case-by-case basis for each specific device. You may refer to Clinical Laboratory Standards Institute (CLSI) document MM3-A2, *Molecular Diagnostic Methods for Infectious Disease* [Ref. 4], for additional information.

4) Extraction Control

The extraction control is an external control that verifies that lysis of MTB-complex isolates and/or subsequent nucleic acid isolation has occurred efficiently. Examples of extraction

controls include a strain of MTB-complex containing the target sequences, or a known MTB-complex positive clinical specimen. The positive control for the complete assay, or the internal control, may serve as an extraction control.

e. Ancillary Reagents

Ancillary reagents are those reagents that an assay manufacturer specifies in device labeling as "required but not provided" in order to carry out the assay as indicated in its instructions for use. Ancillary reagents of concern for this category of device are those that you specify by the catalog or product number, or other specific designation, in order for the device to achieve its labeled performance characteristics. For example, if the device labeling specifies the use of Brand X nucleic acid amplification enzyme, and use of any other nucleic acid amplification enzyme may alter the performance characteristics of the device from that reported in the labeling, then Brand X nucleic acid amplification enzyme is an ancillary reagent of concern. In contrast, if the device involves the use of 95% ethanol, and any brand of 95% ethanol will allow the device to achieve the performance characteristics provided in the labeling, then 95% ethanol is not an ancillary reagent of concern for the purposes of this document.

If the instructions for use of the device specify ancillary reagents of concern, you should include in your submission a description of how you will ensure that the results obtained by testing with the device and these ancillary reagents will be consistent with the performance described in the premarket submission. Every effort should be made to bring the ancillary reagents under the manufacturer's quality system by recommending use of only those ancillary reagents that have been determined to meet the manufacturer's quality standards for the test. This may include application of quality systems approaches, product labeling, and other measures. Your 510(k) submission should address the elements described below:

(1) Use of ancillary reagents in the device risk assessment, including risks associated with the management of reagent quality and variability, risks associated with any inconsistency between instructions for use provided directly with the ancillary reagent and those supplied with the device, and any other issues that could present a risk of obtaining incorrect results with the device.

(2) As part of the risk assessment, your submission should describe how you intend to mitigate risks through implementation of any necessary controls over ancillary reagents. These may include, where applicable:

- User labeling to assure appropriate use of ancillary reagents (see **Section 7.d. - Directions for Use** for further discussion);
- Plans for assessing user compliance with labeling instructions regarding ancillary reagents;
- Material specifications for ancillary reagents;
- Identification of reagent lots that will allow appropriate performance of your device (e.g., if only certain lots of a named ancillary reagents are appropriate for use, these lots should be identified in the labeling);
- Stability testing;

- Plans for Addressing User Technical Questions or Concerns (e.g., telephone Help-Line);
- Corrective and preventive actions;
- Plans for alerting users in the event of an issue involving ancillary reagents that would impact the performance of the device; and
- Any other issues that must be addressed in order to assure safe and effective use of your test in combination with named ancillary reagents, in accordance with the device's instructions for use.

In addition, you should provide testing data to establish that the quality controls you supply or recommend are adequate to detect performance or stability problems with the ancillary reagents.

f. Testing Procedures Using Your Device

You should describe in detail the principles of operation of the device applicable to its intended use. We recommend that you specifically describe testing conditions, procedures and controls designed to provide safeguards for conditions that can cause false positive and false negative results, or present a biosafety risk. These include, but are not limited to, procedures, methods, and practices incorporated into the directions for use to mitigate risks associated with testing (see **Section 7 - Labeling**).

g. Interpreting and Reporting Test Results

Your 510(k) submission should describe how positive, negative, equivocal (if applicable), or invalid results are determined and how they should be interpreted. This description should indicate the cut-off values for all outputs of the assay and include the following information:

- In particular, you should provide the cut-off value for defining a negative result of the assay. If the assay has only two output results (e.g., detected/not detected), this cut-off also defines a positive result of the assay.
- If the assay has an equivocal zone, you should provide cut-off values (limits) for the equivocal zone.
- If the assay has an invalid result, you should describe how an invalid result is defined.

If proper operation of your device involves retesting of equivocal results, you should provide in the device labeling: (1) a recommendation regarding whether retesting should be repeated from the same nucleic acid preparation, a new extraction, or a new patient specimen, and (2) an algorithm for defining a final result by combining the initial equivocal result and the results after retesting. This algorithm should be developed before the pivotal clinical study that evaluates the clinical performance of the assay.

The device labeling should provide recommendations for how the laboratory should follow up any invalid result, i.e., whether the result should be reported as invalid and/or whether re-testing is recommended. If re-testing is recommended, information similar to that for the re-testing of equivocal results (i.e., whether re-testing should be repeated from the same nucleic acid

preparation, a new extraction, or a new patient specimen) should be included in the device labeling.

6. Performance Studies

a. General Study Recommendations

510(k) submissions for this device category should include complete descriptions of the protocols used during assay development in order for FDA to accurately interpret acceptance criteria and data summaries (in tabular form, where applicable) contained in your application. When referring to Clinical and Laboratory Standards Institute (CLSI) protocols or guidelines, we recommend that you indicate which specific aspects of the protocols or guidelines were followed. Relevant findings in published literature may also be cited.

We recommend that you contact the Division of Microbiology Devices prior to initiating clinical studies to obtain feedback regarding planned studies and to confirm that these studies will support the proposed intended use for the device. Additionally, if you are considering device clearance for use in point-of-care settings, you should contact the Division of Microbiology Devices early during device development for additional guidance.

b. Analytical Studies

The analytical studies appropriate for a device of this type will depend on the underlying technology, principles of operation, and available scientific evidence specific to the new device. The following analytical studies are recommended; however, please note that additional analytical studies may be appropriate depending on the specific device characteristics:

1) Nucleic acid extraction

Different extraction methods may yield MTB-complex nucleic acid of varying quantity and quality. Thus the extraction method used with (or as part of) a device can be crucial for successful test performance. Purification of MTB-complex nucleic acid from clinical specimens can be challenging because specimens may contain low mycobacterial loads in the background of human genomic DNA and non-targeted organisms, as well as high levels of proteins and other contaminants.

For these reasons, you should evaluate possible effects of different extraction methods on the performance of the assay if more than one possible extraction method is recommended for use with your device. This should include demonstrating the Limit of Detection (LoD) and reproducibility of your assay with each extraction procedure (see **Section 6.b.(2) - Analytical Sensitivity (Limit of Detection)**) for recommendations for conducting LoD studies). External site studies (including reproducibility and clinical studies) should employ the extraction procedures intended for inclusion in the marketed product labeling. If different sample types are to be studied and included in the intended use of the device, then LoD and reproducibility studies for each extraction method should be conducted on the most challenging specimen type.

We recommend that you perform these evaluations regardless of whether you intend to actually provide reagents for extraction and preparation of nucleic acid in your test kit or you simply recommend the use of the appropriate reagents.

Different extraction methods may be studied at different clinical sites. If you intend to use different extraction methods in your studies, each different extraction method should be evaluated analytically (i.e., by LoD and reproducibility testing) to demonstrate that device performance is similar with the different extraction methods. For example, if three different extraction methods are being studied, the reproducibility study can be designed to evaluate a single extraction method at a single testing site, i.e., extraction method A at site 1, extraction method B at site 2, and extraction method C at site 3. If results do not show equivalence between extraction methods from each clinical testing site (using a different nucleic acid extraction method), the results should be discussed with the Division of Microbiology Devices prior to initiating clinical trials.

If different extraction methods are proposed for use with your device, each method should be performed by at least one clinical site during the clinical performance trials.

2) Analytical Sensitivity (*Limit of Detection)*

You should determine the limit of detection (LoD) of your device using serial dilutions of human isolates of *M. tuberculosis* and *M. bovis.* The isolates should be well characterized strains from recognized culture collections such as the Centers for Disease Control and Prevention (CDC), American Type Culture Collection (ATCC), and German Resource Centre for Biological Material (DSMZ), among others. Testing should be performed for each specimen type included in the intended use.

Your LoD studies should examine possible device variability by testing 3-5 samples over 3-5 days for each serial dilution. The LoD of the device should be estimated as the level of each MTB-complex strain that gives a 95% detection rate. The estimated LoD should be confirmed by preparing at least twenty additional replicates at the LoD concentration and demonstrating that the MTB-complex strain was detected 95% of the time. The recommended reference method for LoD determination is plating and counting bacterial colony forming units (CFU). CFU should be based on colony counts from actual plating and counting of bacteria rather than a theoretical calculation deduced from an estimated cells/mL number (i.e., McFarland units). LoD should also be presented as genomic DNA copy numbers/mL for each dilution.

For studies with respiratory specimens we recommend the use of either MTB-complex negative respiratory specimens or simulated respiratory specimens with serial dilutions of each MTB-complex strain to be studied. A simulated sputum specimen can be contrived using saline solution, mucin, and human cells. If you choose to use a simulated sputum specimen in the LoD study, an analytical specimen equivalency study should be carried out to demonstrate that your assay will generate equivalent results using both the natural specimen and the simulated specimen.

We recommend that you refer to Clinical Laboratory Standards Institute (CLSI) document EP17-A, *Protocols for Determination of Limits of Detection and Limits of Quantitation* [Ref. 5], when designing your LoD studies.

3) Analytical Reactivity (Inclusivity)

You should demonstrate that your device can detect MTB-complex strains representing the global genetic diversity of MTB-complex. The concentration of MTB-complex isolates used in inclusivity studies should be at levels at or near the assay cut-off and should be confirmed by plating and counting bacterial colony forming units (CFU). Isolates used in testing should be well characterized strains from recognized culture collections such as the Centers for Disease Control and Prevention (CDC), American Type Culture Collection (ATCC), and German Resource Centre for Biological Material (DSMZ), among others. Isolate strain characterization should be determined using standardized reference methods (e.g., MIRU-VNTR; Spoligotyping) recognized by a reputable scientific body and appropriate to the strain lineage. Your submission should also cite literature evidence that all species contain the target of interest.

You should demonstrate that your device can detect a minimum of 50 isolates including:

- at least two isolates each of the following:
    - *M. bovis*
    - *M. bovis* BCG
    - *M. africanum* – Clades 1 and 2
    - *M. canetti*
    - *M. microti*
    - *M. caprae*
    - *M. mungii*
- at least two isolates each of the following *M. tuberculosis* strain families:
    - East African-Indian (EAI) – specifically from East Africa, Philippine, and South India
    - Central and Middle Eastern Asian (CAS)
    - Latin American and Mediterranean (LAM)
    - Haarlem
    - S
    - T
    - X
    - U
- at least five isolates from different geographic locations of *M. tuberculosis*, Beijing strain family.

4) Analytical Specificity

(a) Cross-Reactivity

Listed below are microorganisms recommended for testing in cross-reactivity studies. Cross-reactivity studies should include mycobacterial species known to cross react with certain MTB-complex targets (e.g., *M. celatum and M. kumamotonense*), other mycobacterial species related to MTB-complex species, common oral/respiratory tract commensals and pathogens, selected fungi, and selected viruses. You should conduct cross-reactivity studies using a concentration of a minimum 10^8 colony forming units per mL for mycobacteria, fungi and bacteria; a minimum of 10^5 plaque forming units per mL for viruses; and a minimum of 10^6 elementary bodies (EB) or inclusion forming units (IFU) per mL for *Chlamydophila (Chlamydia)*. You should confirm the microorganism identities and you should base the concentrations tested on actual counts and not base them on a theoretical calculation deduced from an estimation (i.e., McFarland units or an absorbance reading).

In silico testing may also be acceptable in some circumstances. We recommend that you consult with the Division of Microbiology Devices early during device development regarding the applicability of in silico testing to your device.

Category 1: Mycobacteria

M. abscessus	*M. leprae*
M asiaticum	*M. malmoense*
M. avium	*M. marinum*
M. celatum	*M. mucogenicum*
M chelonae	*M. scrofulaceum*
M. flavescens	*M. simiae*
M. fortuitum	*M smegmatis*
M. gastri	*M. szulgai*
M. gordonae	*M. terrae complex*
M. intracellulare	*M. thermoresistable*
M. kansasii	*M. trivale*
M. kumamotonense	*M. xenopi*

Category 2: Fungi

Candida albicans	*Aspergillus fumigatus*
C. glabrata	*Blastomyces dermatitidis*
C. krusei	*Histoplasma capsulatum*
C. neoformans	*Penicillium spp.*
C parapsilosis	*Rhizopus spp.*
C. tropicalis	*Scedosporium spp.*

Category 3: Viruses

Adenovirus	*Rhinovirus*
Human Influenzae Virus (Types A and B)	*Rubella Virus*
Human Metapneumovirus	*Rubeola Virus*
Human Parainfluenzae Virus (Types 1, 2, 3)	*Rubula (Mumps) Virus*
Respiratory Syncitial Virus	*Varicella Zoster Virus*

Category 4: Bacteria

Actinomyces israelii	*H. parainfluenzae*	*S. aureus*
A. baumannii	*H. parahemolyticus*	*S. epidermidis*
A. calcoaceticus	*Kingella oralis*	*S. haemolyticus*
B. fragilis	*K. pneumoniae*	*S. lugdunensis*
B. cereus	*K. oxytoca*	*S. equi*
B. subtilis	*Lactobacillus spp.*	*S. pneumoniae*
B. cepacia	*L. pneumophila*	*S. pyogenes*
Chlamydophila	*L. micdadei*	*S. agalactiae*
Chlamydia) pneumoniae	*Leuconstoc spp.*	*S. salivarius*
C. diptheriae	*L. monocytogenes*	*S. maltophilia*
C. jeikeium	*M. catarrhalis*	*Streptomyces anulatus*
C. freundii	*Mycoplasma pneumoniae*	*Veillonella spp.*
Clostridium spp.	*N. sicca*	*Viridans Streptococcus group (a minimum of 5 different species)*
C. pseudodiptheriticum	*N. meningitidis*	*Y. enterocolitica*
E. corrodens	*N. gonorrheae*	*Nocardia asteroides*
Enterobacteriaceae (including ESBL and KPC containing	*N. mucosa*	*N. brasiliensis*

isolates)		
E. aerogenes	*N. lactamica*	*N. otitidiscaviarum*
E. cloacae	*Pediococcus spp.*	*Rhodococcus equi*
E. faecalis	*Peptostreptococcus spp.*	*Tsukamurella*
E. faecium	*P. mirabilis*	
E. coli	*P. vulgaris*	
Fusobacterium spp.	*P. aeruginosa*	
H. influenzae	*S. marcescens*	

(b) Interfering Substances

You should conduct a comprehensive interference study. Relevant interfering substances include, but are not limited to, endogenous substances such as blood and mucus, and exogenous substances such as topical nasal and throat medications and oral medications that may be secreted into respiratory secretions. Potentially interfering substances are presented in Table 2 below. Your interference studies should include, at a minimum, a representative of each listed substance or class.

You should evaluate each interfering substance at its highest medically-relevant concentration ("the worst case") in a simulated matrix with a target concentration close to the assay cutoff; if no significant effect is observed, testing at lower concentrations of the interferent is not necessary.

Please refer to the CLSI document EP07-A2, *Interference Testing in Clinical Chemistry* [Ref. 6], for additional information.

Table 2. Substances/Classes Recommended for Interference Testing

Substance/Class	Active Ingredient
Anesthetics (endotracheal intubation)	Lidocaine
Antibacterial, systemic	Tobramycin, Amoxicillin, Levofloxacin
Antibiotic, nasal ointment	Mupirocin
Anti-tuberculosis drugs	Isoniazid, Rifampin, Pyrazinamide, Ethambutol, Streptomycin
Anti-viral drugs	Zanamivir

Blood (human)	
Bronchodilators	Ephedrine hydrochloride, Epinephrine, Theophylline
Gastric Acid	
Homeopathic allergy relief medicine	Stinging Nettle, Goldenseal, Butterbur, Tea Tree Oil
Human DNA	
Inhaled bronchodilators	Albuterol sulfate, Formoterol, Budesonide
Live intranasal influenza virus vaccine (FluMist®)	Live influenza virus vaccine
Mouthwash/Gargle Solutions	Eucalyptol, Methyl, Salicylate, Thymol, Cetylpyridinium Chloride, Denatured Alcohol
Mucin: Bovine submaxillary gland, type I-S	Purified mucin protein
Nasal corticosteroids	Beclomethasone, Dexamethasone, Flunisolide, Triamcinolone, Budesonide, Mometasone, Fluticasone
Nasal gel (homeopathic)	Luffa opperculata, Sulfur
Nasal sprays or drops	Phenylephrine, Oxymetazoline, Sodium chloride with preservatives
Nebulizing solutions (hypertonic saline)	NaCl (3-5%)
Oral anesthetic/ and Analgesic	Benzocaine, Menthol
Oral Expectorant	Guaifenesin
Physiologic Saline	NaCl (0.9%)
Pneumocystis jiroveci medications	Pentamidine
Specimen Processing Reagents	Cetylpyridinium Chloride (CPC), Oxalic Acid, NAC-PAC™ XPR-plus™ AFB Processing Buffer (Alpha-Tec Systems, Inc.)
Tobacco	
White Blood Cells (human)	

(a) Cut-off and Equivocal Zone

Your submission should describe how the assay cut-off was determined and validated. It is recommended that the cut-off be determined using appropriate statistical methods. For example, you may provide a result distribution, 95th and 99th percentiles, percents of the non-negative (positive or equivocal) results, and other statistical methods, for the clinical specimens without MTB-complex, in your pilot studies. Selection of the appropriate cut-off can be justified by the relevant levels of sensitivity and specificity based on Receiver Operating Curve (ROC) analysis of the pilot studies with clinical specimens. For details about ROC analysis, see CLSI document GP10-A *Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristics (ROC) Plots*) [Ref. 7]. If the assay has an equivocal zone, you should explain how you determined the limits of the equivocal zone. You should validate the performance of your device using the pre-determined cut-off (and equivocal zone, if applicable) in an independent population consistent with the defined intended use of your device.

5) Precision Studies

(a) Within-Laboratory Precision/Repeatability

You should conduct within-laboratory precision studies using the instruments and/or automated components anticipated for use in your clinical study. You may perform these studies in-house, i.e., within your own company facility.

You should evaluate sources of variability (such as operators, days, assay runs, etc.) by testing your device for a minimum of 12 days (not necessarily consecutive), with two runs per day and at least two replicates of each sample per run. In some situations, test days spanning at least two calibration cycles may be necessary. The test panel should consist of at least one *M. tuberculosis* and at least one *M. bovis* isolate spiked in specimen matrix or simulated specimen matrix (provided that you can demonstrate that your device will generate equivalent results using both the relevant specimen matrix and the simulated specimen matrix) at three concentration levels that include:

- Negative sample: a sample with no analyte such that results of repeated tests of this specimen are negative 100% of the time. “
- “Low positive” sample (C 95 concentration): a sample with a concentration of analyte just above the clinical cut-off such that results of repeated tests of this specimen are positive approximately 95% of the time.
- “Moderate positive” sample (e.g., approximately two to three times the concentration of the clinical cut-off): sample with a concentration at which one can anticipate positive results approximately 100% of the time.

CLSI documents EP05-A2 *Evaluation of Precision Performance of Quantitative Measurement Methods* [Ref. 8], and EP12-A2, *User Protocol for Evaluation of Qualitative Test Performance* [Ref. 9], contain further information about designing and performing repeatability studies.

(b) Between Laboratory Reproducibility

The protocol for the reproducibility study may vary slightly depending on the device format. As a general guide, we offer the following recommendations:

- Evaluate the reproducibility of your device at a minimum of three testing sites; this may include one in-house site. At least two of the sites should be domestic sites.
- Use a five day testing protocol (not necessarily consecutive days), including a minimum of two runs per day (unless the assay design precludes multiple runs per day), with three replicates of each panel sample per run. A minimum number of 90 observations should be provided for each test panel sample across the three sites (with at least 30 observations per site for each test panel sample).
- Have at least two operators at each facility perform the test each day.
- Use the same sample panel as described in the within-laboratory precision study above.

CLSI document EP15-A2, *User Verification of Performance for Precision and Trueness* [Ref. 10], contains additional information on reproducibility study design.

6) Specimen Collection, Specimen Storage, and Specimen Shipping Studies

Unless all specimens are expected to be processed as fresh specimens within a specified time frame, you should study device performance under different storage conditions. You should demonstrate that your device generates similar results for the stored specimens at several time points throughout the duration of the recommended storage period and at both ends of your recommended temperature range. The specimen storage studies should include samples close to the cutoff. In addition to the analysis of qualitative results, you should provide analysis of the raw signals (if applicable).

CLSI document MM13-A, *Collection, Transport, Preparation and Storage of Specimens for Molecular Methods* [Ref. 11], contains additional information specific to this topic.

7) Device Shipping and Device Storage Studies

You should evaluate the performance of your device after exposing the device to various shipping and storage conditions that are similar to shipping and storage conditions that you intend to include in your product labeling.

8) Carry-Over and Cross-contamination Study (for multi-sample assays and devices that require instrumentation)

You should demonstrate that carry-over and cross-contamination does not occur with your device. In a carry-over and cross-contamination study, we recommend that you perform an assay run with high positive samples alternating with negative samples in patterns dependent on the operational function of the device. You should perform at least five runs alternating high positive and negative samples. Analyte concentrations in high positive samples should be high enough that 95% or more of the results obtained from specimens of diseased patients in the

intended use population provide a positive result. Negative samples should be samples with no analyte such that results of repeated tests of this specimen are negative 100% of the time.

The carry-over and cross-contamination effect can then be estimated by the percent of negative results for the negative samples that are adjacent to high positive samples in the carry-over study compared to the percent of negative results in the absence of adjacent high positive samples. In addition, you should provide the following information: an analysis of the raw signals (if applicable) in terms of the median of the raw signal of negative samples, the median of the raw signal of negative samples in the carry-over study, a cross-contamination study and difference in the medians along with 95% confidence intervals.

c. Clinical Studies

The clinical performance (i.e., sensitivity and specificity) of your device should be established from a prospective clinical study (or studies) that includes subjects being evaluated for suspected active tuberculosis. You should consult with the Division of Microbiology Devices prior to study initiation regarding proposed studies that may include retrospective or banked specimens to supplement specimens from prospectively enrolled subjects**.**

It is anticipated that sputum will be the most common respiratory specimen type tested with these devices and your study should be statistically powered (see Section 6.c.(5)) to demonstrate acceptable device performance for sputum samples. It is acceptable to combine induced and expectorated sputum samples for analysis purposes. If bronchial specimens are also intended for use with the device, we recommend studying a minimum of 50 bronchial specimens, of which at least 10 should be culture positive and 40 culture negative.[4] Tracheal aspirates are considered comparable to sputum and do not need to be studied independently.

You should establish early during device development whether your device is intended to test respiratory specimens directly or after undergoing specimen processing[5] (i.e., digestion-decontamination-concentration) and clearly state this information in your study protocol. If your device is intended for use both with specimens tested directly and with specimens that have undergone processing, your clinical studies should include both direct and processed specimens.

The specimen processing method(s) intended for use with your device, (e.g., NALC-NaOH, Oxalic Acid, Cetylpyridinium Chloride), should be established early during device development and clearly stated in your study protocol. If more than one method of specimen processing is intended for use with your device, each of these methods should be evaluated during your clinical studies.

There should be at least two specimens tested by the investigational device and reference method per subject. This may be achieved by aliquoting two separate subject specimens or collecting four separate samples from each subject. If both direct and processed respiratory specimens will be tested in the same study, one specimen should be tested directly with your

[4] We recommend that the sponsor describe in a pre-submission the number of patients with non-sputum specimens that will be enrolled in the clinical studies. The use of non-sputum specimen types should be supported by analytical studies showing the different matrix has no effect on device performance.

[5] Throughout this special controls guidance, the term "processed" or "processing" is used to describe the digestion, decontamination, and centrifugation to pellet of respiratory specimens.

device and the other should be tested with your device as a processesed specimen. These respiratory specimens should either be randomized by method (i.e., direct or processed), within subjects, or follow a set sequence (e.g., testing of direct specimen followed by processed specimen for all subjects).

Your clinical study protocol should also clearly describe whether patient specimens will be split for testing between both your device and the reference method, or if multiple patient specimens will be used. Both methods may be permissible within a study depending on specific specimen volume.

In general, the following clinical studies principles should be followed:

- Clinical samples should be collected from a minimum of three geographically diverse locations, one of which should be in the United States.
- Laboratory testing using your device should be performed at a minimum of three different sites representing settings where the device is intended for use. Laboratory testing sites may be the same as the clinical enrollment sites. One laboratory testing site may be the manufacturer's laboratory.
- Reference method testing may be conducted at a centralized laboratory.
- The collection, transport, and testing of specimens to be tested with the investigational device should be performed by individuals with training equivalent to that anticipated for users of the marketed device.
- Clinical enrollment sites and laboratory testing sites should be supervised by qualified principal investigators

1) Reference Method

Your clinical studies should compare the performance of your device to a composite reference method derived from the results of culture/identification, and direct specimen nucleic acid amplification. More specifically, reference method testing should include:

- Mycobacterial culture and isolation using liquid and/or solid media followed by identification of the cultured isolates using either FDA cleared molecular probes, phenotypic culture methods (i.e., conventional biochemical testing combined with growth rate, and colonial morphology), or bi-directional sequencing of the cultured isolates.

  AND

- Direct nucleic acid amplification testing of the respiratory specimen using an FDA cleared or approved assay or other well characterized nucleic acid amplification assay followed by bi-directional sequencing analysis. The sequencing analysis should be performed on both strands of the amplicon, should demonstrate that the generated sequence is at least 200 base pairs of an acceptable quality (e.g., a quality

score of 20 or higher as measured by PHRED or similar software analysis), and should demonstrate that it matches the reference consensus sequence.[6]

We recommend that you refer to CLSI document MM18-A, *Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing* [Ref. 12], for guidelines specific to target sequencing of MTB-complex.

The reference method for tuberculosis positive samples is defined as the detection of MTB-complex by **either** mycobacterial culture/identification **or** direct nucleic acid amplification followed by bi-directional sequencing.

The reference method for tuberculosis negative samples is defined as negative results for **both** mycobacterial culture/identification **and** direct nucleic acid amplification.

Your study protocol should include a description of the specific tests used in the composite reference method determination. If composite reference method testing is done at multiple sites rather than a single central laboratory, your study protocol should describe any differences in the methods used across testing sites. You should describe all quality control measures used for your device and for the tests included in the composite reference method.

While not directly included as part of reference method testing, you should perform acid-fast smears using fluorochrome staining on all respiratory specimens included in the clinical study. These data should be included in your submission for subgroup analysis of device performance based on acid-fast smear status.

It is expected that almost all subjects testing positive with your device will test positive by the reference method (i.e., that your device will have high test specificity). However, we recognize that a small percentage of subjects may be positive by your device but negative by the reference method. This may be possibly due to inhibition of growth of the organism in culture due to the effect of processing on the respiratory specimen, rather than the result being a true false positive. You can address this possibility by adding patient follow-up in the study design and including defined criteria for the diagnosis of clinical tuberculosis [Ref. 13], usually based on clinical and radiographic response to treatment. This can be included as a secondary analysis and may improve the specificity of your investigational device, particularly for devices with high sensitivity.

2) Study Protocols

Your clinical study protocol(s) should be finalized prior to study initiation. The protocol(s) should include complete patient inclusion and exclusion criteria, study procedures, a description of where the tests will be performed, a detailed statistical analysis plan that includes the statistical analysis methods to be used and justification of the study sample size, and other components as appropriate. The study protocol should be explicit regarding whether results from your device will be used for patient management; if so, an Investigational Device

[6] These criteria are based on the Sanger di-deoxysequencing method instruments. If you propose other sequencing methods, you should specify the platform and how you will determine acceptable quality.

Exemption (IDE) may be required for study sites in the US (see Section 6.c.(4) below). You should use FDA data standards for capturing clinical trial data such as CDISC in case report forms and data analysis sets, as appropriate; see http://www.fda.gov/ForIndustry/DataStandards/StudyDataStandards/default.htm for current CDRH data standards.

In the protocol(s) you should describe blinding procedures for both clinicians and laboratory personnel. Operators of your device should not be aware of reference assay results or the results from other diagnostic evaluations (e.g., acid-fast smear results), nor should laboratory personnel performing reference methods be aware of results from your device.

Any planned interim analyses of the study data or examinations of study progress should be described in the clinical protocol/statistical analysis plan. Changes to the study protocol based on examinations of the study data, (e.g., the addition of high prevalence sites due to a lower than expected prevalence of tuberculosis), should be submitted to the Division of Microbiology Devices with a revised protocol.

You should include copies of the original study protocols, and all protocol modifications in your 510(k) submission with any other relevant study information.

We recommend that you contact the Division of Microbiology Devices to request a review of your proposed clinical study protocols while in the development stage as part of the pre-submission review process. For additional guidance related to the clinical protocol, you may also refer to the draft guidance entitled "Design Considerations for Pivotal Clinical Investigations for Medical Devices" found at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm265553.htm.

In addition, your clinical protocol should also address acceptable transport methods, storage conditions, and maximum storage times (if appropriate). Study case report forms should capture any time-sensitive steps, e.g., the amount of time a specimen is stored if not tested immediately. The protocol should also describe safety precautions for the collection, handling, processing, and testing of specimens that will be tested during the study.

3) Study Sites

You should collect respiratory samples from at least three different clinical sites in geographically separate locations. At least one of the sites should be located in the United States.

Your device should be tested at a minimum of three different testing sites representing environments where the device would be used (e.g., clinical laboratories), and by laboratory personnel with training similar to those likely to perform the test in laboratory settings. Testing sites should document all quality control results and all repeat tests for runs with out-of-range quality control values.

Clinical investigations of FDA unapproved and uncleared *in vitro* diagnostic devices, including nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex in respiratory specimens, are subject to the IDE provisions of Section 520(g) of the FD&C Act (21 U.S.C.

360j(g) and the implementing regulations. You should consider how 21 CFR part 812 (IDEs) applies to your particular study and refer to 21 CFR part 50 (informed consent) and 21 CFR part 56 (institutional review board review) for other applicable requirements.

4) Study Populations

Subjects enrolled in your clinical studies should be untreated subjects with suspected active tuberculosis who meet study inclusion and exclusion criteria; the study inclusion and exclusion criteria should match the intended use population of your device. A minimum set of demographic characteristics, including age, gender, HIV status (including CD4 count and viral load if available), the presence of other relevant medical conditions and/or medications, the signs and symptoms of tuberculosis (with date of onset), and radiographic results, should be captured. You should also record the results of the tuberculin skin testing (TST) and interferon gamma release assay (IGRAs), if available.

Due to the unique aspects of studying tuberculosis in children, including the possible use of gastric aspirates for diagnosis, sponsors who wish to include children in their device clinical studies should contact the Division of Microbiology Devices early in device development.

5) Data Analysis and Sample Size

The data analysis plan should clearly specify the primary analysis, which should be based on the specimens collected during the clinical trials. For example, if both a processed and direct specimens were collected, you should report the sensitivity and specificity of each sample type separately along with 95% two-sided confidence intervals and then the sensitivity and specificity of both the direct and processed specimens together along with 95% two-sided confidence intervals. For methods of calculation of confidence intervals, see CLSI EP12-A2 [Ref. 9]. In all cases, the reference method result should be based on at least two specimens.

You should include in the data analysis plan a mechanism to account for all subjects enrolled and for all specimens collected. This information should be provided in the 510(k) submission as well as explanations of all specimens and subjects not included in analysis of the device performance. The 510(k) submission should also include comparisons of device performance against the composite reference method in an appropriate tabular format, and additional analyses for pre-defined patient sub-populations such as those with HIV.

The sample size for your study should be based on the number of subjects providing respiratory samples and not on the number of respiratory specimen collected.
Studies should be powered to achieve an overall sensitivity of approximately 90% with a lower bound of the two-sided 95% confidence interval of 85% [7]. For estimation of sensitivity, approximately 30% of the enrolled subjects should be acid-fast smear negative, and approximately 70% should be acid-fast smear positive. It is anticipated that the point estimate for sensitivity will be approximately 99% in acid-fast smear positive TB subjects and no less than 72% in acid-fast smear negative subjects. Point estimates of device performance (i.e., sensitivity and specificity) for specimen types other than sputum should be similar or superior to results for sputum.

[7] For 200 subjects, 90% (180/200) with 95% two-sided confidence interval: 85.1% to 93.4%

Specificity should be clinically acceptable with the lower limit of a 95% two-sided confidence interval of around 96%.

6) Electronic Data Submission

All study data should be included in your 510(k) submission in an acceptable electronic format; this should include individual patient level data. Data files should include appropriate annotations or separate codebooks and should include all primary and derived variables, e.g., the result of the clinical reference algorithm for determining the presence of MTB-complex. Separate data files for the analytical studies should also be included in the 510(k) submission. Description of the statistical methods applied to the data set should be sufficiently detailed to allow the Agency to reproduce the results reported in the submission from the data files. Manufacturers may refer to the draft guidance entitled "Providing Regulatory Submissions in Electronic Format – General Considerations" for additional guidance related to electronic data submissions, at http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm124751.pdf.

7. Labeling

All *in vitro* diagnostic devices, including nucleic acid-based *in vitro* diagnostic devices for the detection of MTB-complex in respiratory specimens, are subject to statutory requirements for labeling (sections 201(n) and 502(a) of the FD&C Act (21 USC § 321(n) and 352(a),) including adequate directions for use and adequate warnings and precautions (section 502(f) of the FD&C Act (21 USC § 352(f)). Specific labeling requirements for all IVD devices are set forth in 21 CFR 809.10.

Your labeling for devices for the detection of MTB-complex should include information similar to that described below to aid in mitigating the risks identified in Section 4 of this draft guidance document to ensure safe and effective use of these devices.

The requirements in 21 CFR 809.10 should be addressed in device labeling as described below:

a. Intended Use

The intended use statement should clearly specify the intended use of the device, the population in whom the test should be used, and other significant aspects of use as appropriate, e.g., whether the test should be used in conjunction with mycobacterial culture and/or acid-fast smear. The intended use should clearly state that the device is to serve only as an aid in diagnosis and that false negative test results may occur. Additional qualifications may be appropriate based on the results of the clinical studies.

The following statement should be included immediately below the intended use statement:

"The [Insert Test Name] *test should only be performed in laboratories that follow safety practices according to the CDC/NIH Biosafety in Microbiological and Biomedical*

Laboratories[8]*and/or applicable state or local regulations. The* [Insert Test Name] *test must be performed in conjunction with mycobacterial culture to address the risk of false negative results and to recover the organisms for further characterization and susceptibility testing."*

b. Device Description

The Device Description should briefly describe the test methodology used in your device.

c. General Procedure

The General Procedure should include a description of the entire testing process from patient sampling to result reporting.

d. Directions for Use

The Directions for Use should present clear instructions that systematically describe the procedures for using the device and the types of control measures that will minimize risks of inaccurate results. This section of the Labeling should include guidance for biosafety precautions with specimen handling and testing procedures and should clearly specify at which procedural step the test is rendered non-infectious.

Device handling and storage instructions should be included as well as a description of the expiration dating for both open and closed storage conditions for your device and any reagents or other components.

For devices that involve the use of ancillary reagents of concern (see also Section 5(e) of this document), you should:

- Emphasize prominently in labeling that proper product device performance involves use of specific ancillary reagents as directed. This labeling may include warnings against the use of device if specified ancillary reagents are not available.
- Ensure that users can clearly identify which ancillary reagents are suitable for use with your device.
- Ensure that users of your device will understand which instructions for use they should follow when using ancillary reagents that are supplied with instructions for use or other warnings or limitations by the ancillary reagent manufacturer. If there is a conflict between the directions and warnings provided by the manufacturer of the ancillary reagents and the instructions for use that you supply with your device, you should assess and address the risk that users may mistakenly follow the labeling provided directly with the ancillary reagent manufacturer and possibly obtain invalid or inaccurate test results with your device. We note that in some circumstances, statements in the labeling of your device may not be sufficient to address the risks created by this conflict.

e. Quality Control

Your quality control recommendations in the package insert should include a clear explanation of what controls should be used with the assay and the expected results for the control material.

[8] http://www.cdc.gov/biosafety/publications/bmbl5/index.htm

If controls are included with your device, the 510(k) submission should include the specifications for control materials.

f. Warnings, Contraindications, Precautions, and Limitations

All warnings, contraindications, precautions, and limitations relevant to the specific device should be included in the device labeling. At a minimum, you should include a discussion of certain populations where device performance may differ or where the device has not been studied (e.g., pediatrics). Specific precautions regarding the use of specimen types other than respiratory specimens should be included if these other specimen types have not been studied.

If positive and negative interference has been detected or reported for any commonly used collection materials or substances that may be endogenously or exogenously introduced into a specimen prior to testing, you should advise of the possibility of false negative or false positive results as a limitation of your device.

In addition, the following statements should be included as a limitation:

"The performance of the [Insert Test Name] test is dependent on operator proficiency and adherence to procedural directions. Laboratory procedural errors may cause false positive or false negative results. All device operators should have appropriate device training.

A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests."

g. Specimen Collection

Your label should state how specimens should be properly collected, stored, and transported, and that inadequate or inappropriate specimen collection, storage, number of freeze/thaw cycles, and transport are likely to yield false negative test results. Labeling should also state that specimens should be collected as soon as possible after symptom onset and that ongoing treatment may affect device performance.

h. Interpretation and Reporting of Assay Results

You should describe how the operator should interpret each of the possible device results, e.g., positive, equivocal, and negative. You should also describe the recommendations for retesting or reporting of specimens that are equivocal, (if this is a possible device output), or where specimen processing fails (e.g., whether another aliquot of the same specimen or a fresh specimen is necessary). See also Section 5(g). of this document entitled "Interpreting and Reporting Test Results." In addition, you should include the clinical circumstances under which immediate or delayed retesting is indicated.

If appropriate, you should include photographs and/or diagrams to indicate how to interpret results for tests with a qualitative result.

Your labeling should include a statement that tuberculosis is a notifiable disease that must be reported to public health authorities in accordance with state and local law. Additionally, you should indicate that users should verify reporting requirements for their institution, and notify appropriate agencies (e.g., state or local public health departments, the Centers for Disease Control and Prevention) as specified by applicable local and state regulations, if MTB-complex is detected or tuberculosis infection is suspected.

i. Performance Characteristics

Your labeling should include a summary of the study designs and study results described in Sections 5 and 6 of this document that would aid the user in interpreting test results and understanding device performance; this should include descriptions of both clinical and analytical study results.

8. References

1. Centers for Disease Control and Prevention Fact Sheets - *Mycobacterium bovis* (Bovine Tuberculosis) in Humans. Division of Tuberculosis Elimination. September 9, 2011. http://www.cdc.gov/tb/publications/factsheets/general/mbovis.htm.

2. CR Horsburgh, Jr., Priorities for the Treatment of Latent Tuberculosis Infection in the United States. New England Journal of Medicine, 2004;350:2060-7.

3. CDC MMWR, Vol. 60, No. 11 and CDC *Reported Tuberculosis in the United States, 2010*. Atlanta, GA: US Department of Health and Human Services, CDC, October 2011.

4. Clinical and Laboratory Standards Institute. 2006 Molecular Diagnostic Methods for Infectious Disease; Proposed Guideline-Second Edition. MM3-A2. Clinical and Laboratory Standards Institute, Wayne PA.

5. Clinical and Laboratory Standards Institute. 2004. Protocol for Determination of Limits of Detection and Limits Quantitation; Approved Guideline. EP17-A. Clinical and Laboratory Standards Institute, Wayne PA.

6. Clinical and Laboratory Standards Institute. 2005. Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition. EP07-A2. Clinical and Laboratory Standards Institute, Wayne PA.

7. Clinical and Laboratory Standards Institute. 1995. Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristics (ROC) Plots; Approved Guideline. GP10-A. Clinical and Laboratory Standards Institute, Wayne PA.

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10. Clinical and Laboratory Standards Institute. 2006. User Verification of Performance for Precision and Trueness; Approved Guideline-Second Edition. EP15-A2. Clinical and Laboratory Standards Institute, Wayne PA.

11. Clinical and Laboratory Standards Institute. 2005. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline. MM13-A. Clinical and Laboratory Standards Institute, Wayne PA.

12. Clinical and Laboratory Standards Institute. 2008. Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline. MM18-A. Clinical and Laboratory Standards Institute, Wayne PA.

13. American Thoracic Society, CDC, and Infectious Diseases Society of America. Treatment of Tuberculosis. American Journal of Respiratory and Critical Care Medicine (2003) 167:603 - 62.